Frequently Asked Questions About HPLC-ECD

Listed below are some of the most frequently asked questions received concerning Electrochemical detection with HPLC.

| <u>Noisy baseline</u> | <u>Cleaning Cells</u> | <u>Shutdown Precautions</u> | <u>Long Term</u> | <u>Overnight</u> | <u>Filtering Mobile Phase</u> | | <u>Baseline Fluctuations</u> | <u>pH Effects</u> | <u>Maximum Response</u> | <u>Nitric Acid Cleaning</u> | <u>Changing Filters</u> | <u>Valve</u> Troubleshooting |

What should I do if my baseline is noisy (fluctuating) and/or unstable (drifting).

Noisy or unstable baselines are typically associated with high background currents which can be caused by many factors. Below is a list of some possible causes of high background currents within an high performance liquid chromatography electrochemical detection (HPLC-ECD) system as well as some causes of noise that may not be due to high background currents. Keep in mind that this list includes only a few of the possible causes. Remember there are no specific background currents that a cell should generate. The background current is a result of assay conditions, system performance, cell age, and treatment of the cell.

Common sources of high background currents:

WATER! Water is extremely important in an HPLC-ECD system. Water must be of 18 megaohm-cm resistance or higher. It is then recommended that this water be subjected to further cleaning, by a procedure called water polishing. (Refer to ESA Technical Note "Water Polishing" p/n 70-1668).

Contaminated or impure chemicals used to make the mobile phase and/or preparation of samples. The samples themselves also may be a source of contamination. Alumina or material from extractions may enter the HPLC system and contaminate the column and electrodes.

EC active chemicals within the mobile phase, (e.g., EDTA, TEA). EDTA is commonly used in mobile phases to chelate oxidizable metals, thereby lowering background currents. This compound is highly EC active at >400mV. If working at > 400mV you should not use EDTA in the mobile phase; use citrate instead. Triethylamine (TEA) is used to improve chromatographic peak shape. This additive tends to contain electroactive impurities which may oxidize typically at high potentials. Use minimal amounts of TEA, typically <100µL/L or base-deactivated HPLC columns.

Stainless steel tubing within the system may corrode. The release of contaminants or the presence of corrosion sites within the system can cause cell fouling and/or high background currents. Customers with stainless steel tubing are advised to passivate their system regularly or use PEEK tubing. This tubing can be purchased directly from ESA.

A ruptured pulse damper can leach contaminants into the system typically causing increased background currents and/or pressure to rise over time.

Other sources of noise or drift:

Dissolved gases in the pump head. Fluctuation in pump pressure is normally associated with this problem. Ensure that the mobile phase is properly degassed. Stop the pumps, allow the pressure to bleed off and then purge the pump(s) for 30 second. Degas mobile phase prior to running on the system (For further details see item 6 below).

Dissolved gases in the detector cell. Ensure that the mobile phase is thoroughly degassed.

Specific to cells with purge ports (for cell Model#s 5014B and 5041) is the possibility that the cell was not purged properly before use.

📕 Page Top

How can I clean my cell?

Refer to Technical Note "Prolonging Cell Performance" (p/n 70-5017). This contains both instructional text and technical references for keeping cells running at optimum performance levels.

When I shut down my system what precautions should I take?

Turn off the cells. Flush the entire system with appropriate wash solution (see NOTE below). Turning off the power to each device in the system is not absolutely necessary as long as the cells are turned off and the flow on the pump has been stopped. For long-term storage it is recommended to turn the power off at each device.

NOTE. The shutdown procedure for an HPLC-ECD system may be different depending on the length of time for shutdown. Below is a list of considerations that should be taken into account when establishing a proper shut down procedure.

Overnight shutdown

Isocratic system – Remember that if you are using the system on a daily basis it may be advantageous to leave your EC system running. In this case you would typically re-circulate your mobile phase at a reduced flow rate (0.1mL/min) with cells off (see item 4 below).

Gradient system – With a gradient system you cannot recirculate. Instead you should set your flow to 50% A, 50% B at a total flow rate of 0.025mL/min. Run the system to waste.

Long term shutdown

Flush the system thoroughly to remove salts. It is recommended that this be done using a solution that is identical to your running mobile phase without the buffer or salt additives (typically the organic modifier and water). Caution! Water alone or dilute concentrations of organic modifier if left in an HPLC system for long periods of time may lead to microbial growth and cause a variety of problems. Water can also cause problems with silica based reversed phase columns and should not be used by itself for long periods of time (>20 to 30min) when a column is in-line. After flushing out the salts it is recommended that the column be flushed with the storage solvent recommended by the column manufacturer and then removed and capped so it will not dry out. The system may also be capped off if storage is going to exceed several months.

NOTE: For most EC applications buffer salt concentrations range from 50 – 100mM. Without flow, precipitation of these salts may occur in the HPLC system. The precipitate can cause high backpressure and ghost peaks as well as other problems. In addition, an aqueous solution without an organic modifier (e.g. methanol) is prone to microbial growth. In order to discourage microbe growth ESA recommends using a microbicide such as reagent MB (PN 70-1025).

Page Top

Why do I have to filter my mobile phase and samples through a 0.22 µm pore sized nylon filter?

There are two main reasons for filtering through a 0.22 µm filter.

The first reason is to remove particulates from the mobile phase to reduce the chance of column blockage. The second reason for filtering is that the working electrodes within your coulometric cells are made of a porous graphite material. The pore size of the graphite is approximately $0.2 \ \mu$ m. To protect the cells from becoming clogged due to particulates we strongly recommend filtering through a $0.22 \ \mu$ m filter. In addition, filter elements are placed in front of each cell. Like filtering the purpose of these in-line filters is to protect the cells from particulates. The filter elements are relatively inexpensive and easy to replace if they become clogged.

- Filter Holder (PN 70-0893)
- Graphite filter elements before the injector (PN 70-0898)
- Peek filter elements after the injector (PN 70-3824)

Is it OK to leave the potentials "On" overnight?

Yes, as long as mobile phase (that contains buffer salts) continues to flow through the HPLC-ECD system. Depending on the time needed for equilibration some users leave the system running. By leaving the system running there is no equilibration time needed before running samples. If the pumps fail during the night however, having potential applied with no flow can damage the cells. It should be noted that if you do not require low sensitivity, and if the time of equilibration is short, you may want to turn the cells off or reduce the potential to 0mV when not running. This can increase the life of the cell by reducing the time that the cell is on, and drawing current, especially when running at high potentials.

📕 Page Top

What do I do when my baseline fluctuates in a cyclic manner?

When baselines fluctuate in a cyclic manner the baseline can be described as a saw tooth. All too often noise problems are immediately blamed on the cell. In fact it is extremely rare for the EC cell to cause cyclic noise. In most cases this noise indicates a problem with the pump. To test if the pump is causing the problem measure the distance between the up and down fluctuations (crest to crest) of the noise. Now cut your flow rate in half. Again observe the distance between fluctuations. If the pump is the problem then the distance between cycles will double. Cyclic pump noise can be caused by different factors, here are four common causes:

- 1. Stuck check valve consult pump manual.
- 2. Air in the pump head prime and purge pump to remove air from the system.
- 3. Clogged mobile phase inlet or obstructed inlet tubing.
- 4. Limitations of the pump*

* **NOTE:** Due to the design of certain pumps their ability to produce a pulseless baseline when measured at high sensitivity will vary from pump to pump. EC detection is much more sensitive than UV to pressure fluctuations and a pulseless baseline on the UV does not necessarily mean the pump is sufficient for high sensitivity EC applications. To help with cyclic noise a pulse damper may be installed after the pump to reduce the amount of baseline fluctuation.

CAUTION! Consult with pump manufacturer before you install a pulse damper. Certain pumps cannot be used with a pulse damper.



What effect does pH have on cell response?

In most cases a low pH (acidic environment) will tend to prevent some analytes from oxidizing. At lower pH, the potential needed to oxidize an analyte may need to be higher than when measured at neutral or basic pHs. The reverse can also be correct in that a higher pH or (basic environment) will tend to promote oxidation.

NOTE: In most cases low pH is used to keep analytes (e.g., catecholamines) from auto-oxidizing on their own. It is important to realize that altering pH will also affect separation chemistry, most notably the elution order of compounds separated on the column. In general do not concentrate on pH to affect a higher response at the electrode. Instead, develop your chemistry so that you achieve the separation needed while minimizing auto-oxidation. Analyte response can then be optimized by constructing a current-voltage (CV curve). Information for performing CV curves is included in question #8 of this document.

How do I determine the best potential to produce a maximum response?

NOTE: This procedure assumes constant chromatographic conditions. (i.e. a stable chromatographic method with acceptable analyte separations). This procedure also requires the operator to possess basic knowledge of Coulochem operation, EC theory, and an understanding of CV curves. For reference on hydrodynamic voltammograms (a.k.a. CV curves) see (sec. 6.3, 6.4a, and 6.4b) in the Coulochem II operating manual.

- 1. Set up the HPLC-EC system with all conditions as outlined per assay of interest.
- 2. Turn on cells, apply potential to electrode of interest to 100 mV higher than in the published assay or application and let equilibrate.
- 3. Set the potential (E) to 200mV on the channel that you are not optimizing (Remember you are optimizing one electrode at a time. Setting an electrode to 200mV essentially removes any chance of the compound oxidizing on that electrode).
- 4. Perform an injection of a standard of a known concentration.

NOTE: Standard should be fairly concentrated (i.e. 10 to 50 ng on column) you do not want to run this procedure at or near your limit of detection. Also, it is important to run this first standard at a potential which you believe will produce close to a maximum response.

5. Adjust gain or R-value on the Coulochem so your peak or peaks of interest run about 70 - 80% of the range you have chosen.

NOTE: Adjust gain so peak is large and visible but not off-scale. Once gain or R-value is set, it should remain constant throughout the procedure.

6. Next, apply a potential (E) on the electrode of interest to 50mV less than the prior injection. Allow baseline to stabilize and autozero the detector.

7. Once baseline is stable inject std. Make sure chromatogram is complete and acceptable. Record height or area result.

8. Repeat steps 6 and 7 decreasing the potential (E) in 50mV increments until no area or peak height is observed.

9. Plot peak height (Y- axis) versus detector potential (X-axis) for each compound of interest.

10. For your method, choose the lowest applied potential for the channel of interest where no further increase in peak height or area occurs with additional higher applied potentials (i.e. if maximum peak response was achieved at 450, 500 and 550mV, choose 400mV as your working potential). If you have a preceding screening electrode, set that electrode' s potential to the highest potential where no peak

response was observed. The idea of this exercise is to set the two electrode as close together as possible, thereby minimizing the applied potential window while still maximizing the peak response.

Page Top	
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What are the pH operating ranges for my cells?

Operating at pH ranges (1-12) for the most part will not damage the cells or their components. However, special consideration must be taken when using extreme pHs. Be aware that a combination of basic pH and high background current may damage the cell. Secondly, it is important to note that the limiting factor for pH will typically be the column. Ensure that your column is compatible with the pH of your chemistry. Using a chemistry outside the pH range for your column can cause column packing to be released into the system thereby contaminating or damaging cells.

How often should filter elements be changed?

Under ideal chromatographic conditions changing the filter elements should not be necessary, however in practice the filters do become clogged and must be replaced periodically. The frequency of replacement is dependent on the level of particulate matter in the mobile phase and samples that are put into the system.

An increase in pressure is an indication that it may be time to change a filter. Keeping a daily log of system pressure is highly recommended. To check if a filter is the cause of high-pressure start with the first filter in-line on the system. WAIT FOR THE PRESSURE TO DROP. DO NOT DISMANTLE AN HPLC SYSTEM WHEN THE SYSTEM IS UNDER PRESSURE. Disconnect the tubing after the filter housing. Once disconnected turn on the flow and allow outlet of filter holder to flow to waste. Record the pressure and then remove the filter. Again record the pressure then subtract the second reading from the first. If the difference in pressure is greater than 8-10 bar or 100psi, the filter element should be replaced. Do this for the rest of the filters in the system.

CAUTION!! Be sure the cells are off and the system pressure is below 10 bar before disconnecting the filter.

If the in-line filters need to be changed frequently further investigation is warranted. Depending on which filters become clogged you can use this information to find the source of the particulates. If the filter after the pump needs frequent changing then the mobile phase (including microbial growth) or pump seal deterioration are the most likely the cause. If the filter after the injector needs frequent changing then the column is likely to be the problem. Although these are very good guidelines keep in mind that these are by no means absolute. Common sense and logic must prevail when troubleshooting any HPLC system for pressure problems.



Should I use nitric acid to clean the cell?

Cell cleaning with nitric acid is not recommended. Only use nitric acid to clean the cell as an absolute last resort. Never expose the column to nitric acid. Cell cleaning is outlined in detail in the Coulometric Sensor Technical Note 70-1989. Never have the potential applied to the cells when treating them with nitric acid. The use of nitric acid in the cells <u>VOIDS</u> all cell warrantees. 6N nitric acid can be used to clean or passivate stainless steel components of an EC-HPLC system. Consult section 8.5 of the Coulochem II operating manual for instructions on cleaning the cell with nitric acid. If you are using nitric acid to passivate stainless steel components within your HPLC system, please consult section 4.3 of the Coulochem II Operations manual.

Valve Troubleshooting

Imprecision in the response of peaks between injections could be due to problems with the injection method or the injector. For manual injector troubleshooting information, please go to www.rheodyne.com.

